Cont'd entitled "High Throughput DNA Sequencing Vector" (GENSET.015A, Serial No. 09/058,746, now U.S. Patent Number 6,022,716), the disclosure of which is incorporated herein by reference.

Please replace the paragraph beginning on page 20, line 20 with the following paragraph:

Alternatively, BAC subcloning may be performed using vectors which possess both a high copy number origin of replication, which facilitates the isolation of the vector DNA, and a low copy number origin of replication. Cloning of a genomic DNA fragment into the high copy number origin of replication inactivates the origin such that clones containing a genomic insert replicate at low copy number. The low copy number of clones having a genomic insert therein permits the inserts to be stably maintained. In addition, selection procedures may be designed which enable low copy number plasmids (i.e. vectors having genomic inserts therein) to be selected. In a preferred embodiment, BAC subcloning will be performed in vectors having the above described features and moreover enabling high throughput sequencing of long fragments of genomic DNA. Such high throughput high quality sequencing may be obtained after generating successive deletions within the subcloned fragments to be sequenced, using transposition-based or enzymatic systems. Such vectors are described in the U.S. Patent Application entitled "High Throughput DNA Sequencing Vector" (GENSET.015A, Serial No. 09/058,746, now U.S. Patent Number 6,022,716), the disclosure of which is incorporated herein by reference.

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Please replace the paragraph beginning on page 21, line 19 with the following paragraph:

As a preferred alternative to sequencing the ends of an adequate number of BAC subclones, the above mentioned high throughput deletion-based sequencing vectors, which allow the generation of a high quality sequence information covering fragments of ca. 6kb, may be used. Having sequence fragments longer than 2.5 or 3kb enhances the chances of identifying biallelic markers therein. Methods of constructing and sequencing a nested set of deletions are disclosed in the U.S. Patent Application entitled "High Throughput DNA Sequencing Vector" (GENSET.015A, Serial No. 09/058,746, now U.S. Patent Number 6,022,716), the disclosure of which is incorporated herein by reference.

C3

Appl. No. : 09/463,075
Filed : January 14, 2000

Please replace the paragraph beginning on page 49, line 27 with the following paragraph:

In practice this microsequencing analysis is performed as follows. 20 µl of the microsequencing reaction is added to 80 µl of capture buffer (SSC 2X, 2.5% PEG 8000, 0.25 M Tris pH7.5, 1.8% BSA, 0.05% TWEEN® 20 (polyoxyethylene (20) sorbitan monopalmitate) and incubated for 20 minutes on a microtiter plate coated with streptavidin (Boehringer). The plate is rinsed once with washing buffer (0.1 M Tris pH 7.5, 0.1 M NaCl, 0.1% Tween 20). 100 µl of anti-fluorescein antibody conjugated with phosphatase alkaline, diluted 1/5000 in washing buffer containing 1.8% BSA is added to the microtiter plate. The antibody is incubated on the microtiter plate for 20 minutes. After washing the microtiter plate four times, 100 µl of 4-methylumbelliferyl phosphate (Sigma) diluted to 0.4 mg/ml in 0.1 M diethanolamine pH 9.6, 10mM $MgCl_2$ are added. The detection of the microsequencing reaction is carried out on a fluorimeter (Dynatech) after 20 minutes of incubation.

Please replace the paragraph beginning on page 52, line 8 with the following paragraph:

Examples 15-23 illustrate the application of the above methods using biallelic markers to identify a gene associated with a complex disease, prostate cancer, within a ca. 450 kb candidate region. Additional details of the identification of the gene associated with prostate cancer are provided in the U.S. Patent Application entitled "Prostate Cancer Gene" (GENSET.018A, Serial No. 08/996,306, now U.S. Patent Number 5,945,522), the disclosure of which is incorporated herein by reference.

IN THE CLAIMS:

Please cancel Claims 87, 90 and 91.

Please amend Claims 86, 88-89, and 92-105 as follows:

86. (Amended) A method of obtaining a plurality of single nucleotide polymorphisms comprising the steps of:

(a) obtaining a human nucleic acid library comprising a plurality of genomic DNA fragments containing the full human genome or a portion of the human genome comprising at least 100kb;